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### A NOTE ON TWO FACTORS AFFECTING THE SERO-DIAGNOSIS OF SYPHILIS

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THE two factors referred to are (1) contamination of patients' blood with alcohol (methylated spirit), (2) employment of heterophile antigen.

The following abbreviations are used :—

H.H.	= Human Heart Extract.
S.H.	= Sheep Heart Extract.
G.P.H.	= Guinea-pig Heart Extract.
W.R.	= Wassermann Reaction.

#### (1) CONTAMINATION OF PATIENTS' BLOOD WITH ALCOHOL (METHYLATED SPIRIT)

It may not always be realised sufficiently that, when needles and syringes employed for withdrawing blood for serological tests for syphilis are kept in methylated spirit, this must be scrupulously removed before use.

The experiments described below were undertaken to ascertain the effect upon the complement fixation and flocculation (Sigma) tests for syphilis of contamination of the patient's blood with spirit at the time the specimen is taken.

In the table eight experiments are set out, from which it is seen that the reacting power of serum is markedly reduced for the Wassermann test and Sigma test (and probably, therefore, also for other flocculation tests) by traces of spirit, and that in this way an otherwise " doubtful " or weakly positive reaction might easily be rendered negative, whilst an otherwise moderately strong reaction might become weak or doubtful.

The method adopted was to bleed known, probably Wassermann positive, cases of syphilis into a series of test tubes containing various quantities of spirit. The

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depth of coloration (hæmolysis) of these experimental sera produced by the action of the alcohol was in no instance deeper than in specimens which are submitted to laboratories from time to time in the ordinary course of routine work.

That hæmolysis *per se* does not reduce the reacting power of the serum was ascertained by tests in which nine syphilitic bloods were received, each into two tubes, in one of which hæmolysis was produced mechanically by breaking up the clot. The degree of hæmolysis so caused equalled that of any of the alcoholised specimens. The hæmolysed and unhæmolysed fractions were then tested in parallel in varying serum dilutions similarly to those set out in the table.

No. of Specimen.	Quantities of Methylated Spirit and Blood.			W.R. with primary serum dilutions as under: 1 volume.					Sigma Units.
	Spirit in c.c.	Blood in c.c.	Approximate Proportion.						
I.				1/5	1/10	1/20	1/40	1/80	
	—	5	—	+ ±	± ±	±	—	—	9·1
	0·1	5	1 in 51	+ ±	±	±	—	—	5·4
	0·3	5	1 in 18	±	±	—	—	—	Not tested.
II.				1/5	1/10	1/20	1/40	1/80	
	—	5	—	+ ±	+ ±	±	—	—	—
	0·1	5	1 in 51	+ ±	+ ±	±	—	—	—
	0·3	5	1 in 18	± ±	±	—	—	—	Not tested.
III.				1/5	1/10	1/20	1/40	1/80	
	—	5	—	+ +	+ +	+ +	+ +	+ +	54 +
	0·1	5	1 in 51	+ +	+ +	+ +	+ +	+ ±	44 +
	0·3	5	1 in 18	+ ±	± ±	±	—	—	Not tested.
IV.				1/5	1/10	1/20	1/40	1/80	
	—	5·5	—	+ +	+ +	+ ±	±	—	16·6
	0·1	5	1 in 51	+ +	+ +	±	—	—	3·2
	0·3	4·5	1 in 16	± ±	—	—	—	—	Not tested.

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No. of Specimen.	Quantities of Methylated Spirit and Blood.			W.R. with primary serum dilutions as under: 1 volume.				Sigma Units.
	Spirit in c.c.	Blood in c.c.	Approximate Proportion.	1/5	1/10	1/20		
V.	—	4·8	—	+	+	±		3·8
	0·1	3·0	1 in 31	+	±	±	—	1·8
	0·3	4·75	1 in 17	—	—	—		Not tested.
VI.	—	4·5	—	+	+	+	+	54 +
	0·1	4·8	1 in 49	+	+	+	+	34 +
	0·3	5·5	1 in 19	+	+	±		Not tested.
VII.	—	4·5	—	No W.R.				23
	0·05	4·8	1 in 97	,,				16·3
	0·1	4·5	1 in 46	,,				13·7
	0·15	4·6	1 in 32	,,				8·1
VIII.	—	3·6	—	+	±	±	±	No Sigma test.
	0·05	4·6	1 in 93	+	±	±	±	,,
	0·1	3·7	1 in 38	±	±	±		,,
	0·15	5	1 in 34	±	±	—		,,

## *Remarks on Table I*

(1) In the Wassermann test each serum was tested with three and with five doses of complement.

++ = complete inhibition of lysis with both doses.

+ ± = complete inhibition of lysis with three ; partial with 5 doses.

+ ± = complete inhibition of lysis with three ; partial with five doses, but less than + ±.

± = partial inhibition of lysis with three ; none with five doses.

And so on.

(2) Control tubes in which saline was substituted for spirit in a proportion equal to the highest concentration

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of spirit showed that the diluting effect of the spirit in weakening the reactions was negligible.

(3) A proportion of spirit in blood of 1 in 18 caused solidification of the serum in the course of the ninety minutes' inactivation for the Sigma test. The thirty minutes' inactivation for the Wassermann test at the same temperature had no such effect.

(4) Specimen II., without addition of spirit, gave a negative Sigma result.

(5) A " + " sign after the number of Sigma units signifies that the end-point was not reached in the fifth tube and that further dilutions were not put up. Thus 54+ signifies " total " flocculation, and 44+ " total minus " flocculation in the fifth tube.

### (2) EMPLOYMENT OF HETEROPHILE ANTIGEN IN THE W.R.

In certain laboratories extract of guinea-pig heart is still employed in the W.R.

As was originally shown by Forssman,<sup>1</sup> a hæmolysin for sheep's red blood corpuscles may be produced by the injection of guinea-pig organs into rabbits. Subsequently Browning<sup>2</sup> and Taniguchi<sup>3</sup> drew further attention to this phenomenon, and emphasised that the employment in the W.R. of an antigen made from guinea-pig heart constitutes a danger in that false positive reactions may be elicited. For since the normal hæmolysin content of human serum is of heterophile type and bears no relation to syphilitic infection,<sup>2</sup> if a guinea-pig heart extract (heterophile antigen) is used to test a serum the natural hæmolysin content of which is sufficiently high, the necessary conditions for the binding of complement are established through the interaction of heterophile antigen and heterophile antibody, even though the serum be non-syphilitic. This is well shown in some experiments by Browning,<sup>2</sup> and the following case, shortly summarised, also illustrates the point. A serum gave a clean negative result in the W.R. when tested with human heart extract (and also—see below—with an extract of sheep's heart), but a positive result when tested at the same time in exact parallel with guinea-pig heart extract of proved antigenic efficiency. Complement titrated in parallel with the three extracts gave the same titre. The Sigma

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test was negative, and the patient showed no signs of venereal disease past or present. Tested for content of hæmolyisin one volume of serum diluted 1 in 5 gave complete lysis of one volume 3 per cent. blood corpuscles after five minutes' incubation in the water bath at 37° C. in the presence of one volume of saline and one volume of complement diluted 1 in 10. A complement control failed to produce any lysis whatever in thirty minutes. Two further non-syphilitic sera, tested at the same time, gave clean negative results with both H.H. and G.P.H. Tested for hæmolyisin content, they gave partial lysis in thirty minutes under the conditions detailed. Similar results have been obtained by the writer from time to time with the experimental use of G.P.H.

That the non-specific fixation is related to the natural hæmolyisin content of the human serum is shown by the fact that when this is absorbed out of the serum prior to test, G.P.H. gives the same results as H.H.<sup>3</sup>, provided, of course, that the two extracts are otherwise of equal value.

Since, as referred to above, human serum normally contains hæmolyisin for sheep cells, it might be supposed that the use of an antigen in the W.R. made from sheep heart (as in some laboratories) could lead to non-specific fixation.

It is, of course, well established that S.H. does not act as a heterophile antigen (although sheep corpuscles do so<sup>2 3</sup>), and that reaction of heterophile antibody (*e.g.*, the normal hæmolyisin contained in human serum) with heterophile antigen is strictly specific and does not occur with non-heterophile antigen. Nevertheless, it seemed worth while to test a single sheep heart antigen from this point of view in the W.R.

As was to be supposed, it was found to act quite normally in such a test. Four non-syphilitic sera which gave a slight degree of fixation ( $\pm$  to  $\pm \pm$ ) with G.P.H. gave a clean negative result with H.H. and S.H., whilst a fifth, giving  $\pm \pm$  with G.P.H., gave  $\pm$  with both H.H. and S.H. A sixth non-syphilitic serum has already been referred to with which G.P.H. gave a definite positive result and H.H. and S.H. a clean negative.

That the S.H., which had the same anti-complementary titre as the H.H. and G.P.H., was efficient in antigenic power was shown by the identical results obtained in a W.R. with six weakly reacting syphilitic sera when tried

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in parallel with the H.H. ; two gave +  $\pm$ , one gave  $\pm$   $\pm$ , two gave  $\pm$ , and one gave  $\pm$ .

It may be added that, while G.P.H. is that which has been most commonly employed in the sero-diagnosis of syphilis, among the organic extracts that act as heterophile antigens, horse heart extract (heterophile) is used in the Meinicke and in the Vernes flocculation tests. As is well known, extracts of heterogenetic tissues may be flocculated by non-syphilitic human serum <sup>3</sup> and this has been referred to again recently by Hooker <sup>4</sup> in connection with the use of horse heart extract.<sup>5</sup> According to Forssman <sup>1</sup> it is less active in binding heterophile (guinea-pig-rabbit) antibody than is guinea-pig tissue (kidney).

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